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Multiple Resistance in the Larger House Fly *Musca domestica* in Germany†

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Abstract: Populations of the housefly *Musca domestica* isolated from farms in different German districts with strong resistance problems were compared to laboratory strains with varying resistance spectra. Resistance against pyrethroids, organophosphates and carbamates was tested using impregnated filter papers, and by topical application using a susceptible housefly strain (origin WHO) for comparison. The multi-resistant fly strains tested had a strong resistance against these insecticide groups, ranging from 37- to >10 000-fold for organophosphates and 150- to >6600-fold for pyrethroids. The constituent enantiomer pairs of the α-cyano-pyrethroid cyfluthrin were tested, as was beta-cyfluthrin. With respect to multi-resistant fly strains, the isomers **II** and **IV** had the best activity, with LD_{50} values of 0·012 and 0·014 μg per fly, respectively. In addition, different groups of insect growth regulators (juvenile hormone analogues, chitin synthesis inhibitors and one triazine derivative) were tested in a special larvicidal test. The chitin synthesis inhibitors were quite effective against multi-resistant *M. domestica* strains except for one strain with strong resistance against chitin synthesis inhibitors, developed after extensive treatments with benzoylphenylureas for several years. The fly strains tested were not resistant against cyromazine. Additionally, the insecticides were combined with the synergists piperonyl butoxide, tributylphosphorotrithioate (DEF) and Cibacron blue and tested against the fly strain with the strongest resistance spectrum ('Grimm') in comparison to the susceptible strain ('WHO-N'). Piperonyl butoxide had the greatest effect on the efficacy of cyfluthrin followed by Cibacron blue and DEF. In a parallel investigation with susceptible and resistant house fly strains, different enzyme activities related with resistance mechanisms were tested, e.g. glutathione *S*-transferase (3·5-fold) and mixed-function oxidase (2·3-fold). Implications of these results for management of insecticide resistance in *M. domestica* are discussed.

Key words: *Musca domestica*, resistance, organophosphates, carbamates, pyrethroids, insect growth regulators, isomers, synergists, mixed function oxidases, glutathione *S*-transferases, esterases, acetylcholine esterase inhibitors

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1 INTRODUCTION

The house fly, *Musca domestica* L., is one of the most important hygiene pests worldwide, its ability to carry diseases making it a serious pest.[1] The first effective insecticides (e.g. DDT and lindane) were launched against flies in the 1940s. However, their effectiveness became unsatisfactory within a few years as fly populations developed resistance against these insecticides. This has been the case with all new insecticides introduced against flies since then.[2–4]

Resistance against insecticides has been found in at least 400 arthropod species.[5] For important pest species, such as *M. domestica*, populations with resistance against nearly all common insecticides have been identified. This resistance is due to stable specific gene expression and is found in larvae as well as in adults. Resistance in other species has a similar genetic basis (e.g. *Tribolium castaneum* Hbst., *Lucilia cuprina* Wied. and *Drosophila melanogaster* Meig.).[6] There are a limited number of genetic factors involved in development of resistance which are not strictly bound to specific insecticide molecules. This is a reason for the partial occurrence of cross-resistance to novel insecticidal groups.[7] Several types of resistance have been detected so far, including target-site resistance, metabolic resistance, decreased penetration and sequestration.[8,9]

For most types of resistance, synergists have been described which can at least partially restore the insecticidal activity.[10,11] Mostly, these compounds are inhibitors of metabolic enzymes, e.g. piperonyl butoxide as an effective mixed function oxidase inhibitor or tributylphosphorotrithioate as an inhibitor of esterases.[12,13] The mechanism of resistance can be concluded from an examination of the activity patterns with specific synergists.[10]

With the characterisation of the resistance mechanisms of specific house fly populations and the determination of appropriate synergists, an insecticide resistance management strategy can be developed. The characterisation of resistance mechanisms is important to devise solutions to deal with multi-resistant fly populations and to develop effective fly control. This article should help to identify the resistance spectrum of German house fly populations.

2 EXPERIMENTAL METHODS

2.1 Chemicals

2.1.1 Insecticides

cyfluthrin (*RS*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*, 3*RS*; 1*RS*, 3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

beta-cyfluthrin A mixture of two enantiomeric pairs: A **II** (*S*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*R*, 3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate + (α*R*) (1*S*, 3*S*); **IV** (α*S*) (1*R*, 3*S*) + (α*R*) (1*S* ,3*R*). (See Reference 21)

trichlorfon dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate

fenitrothion *O*,*O*-dimethyl-*O*-4-nitro-*m*-tolylphosphorothioate

phoxim *O*,*O*-diethyl α-cyanobenzylideneamino-oxyphosphonothioate

coumaphos *O*-3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yl *O*,*O*-diethyl phosphorothioate

propoxur 2-isopropoxyphenyl methylcarbamate

triflumuron 1-(2-chlorobenzoyl)-3-(4-trifluoromethoxyphenyl) urea

pyriproxyfen 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether

cyromazine *N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine

2.1.2 Synergists

piperonyl butoxide (PBO) 2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether

Cibacron blue anthraquinone dye, colour index 'Reactive blue 2'

2.1.3 Other compounds

S,*S*,*S*-tributyl phosphorotrithioate (DEF)

All chemicals were more than 98% pure.

2.2 Target insects

The insecticides were tested against four populations of *M. domestica* with different resistance spectra. The susceptible *M. domestica* strain 'WHO-N' was isolated in the 1950s and cultured by the University of Padua (Italy). Since 1963 this strain has also been cultured in our laboratories. The *M. domestica* strain 'Hans' was taken from a farm situated in the lower Rhine district in 1980 when pyrethroid resistance started. The flies were challenged with the pyrethroid cyfluthrin in a six-month rhythm. The 'Reichswald' strain was isolated from a pig farm with extreme fly problems in the lower Rhine district (Germany, March 1990) and the 'Grimm' strain from a farm with similar fly problems in November 1994. The 'Grimm' strain is characterised by a high resistance against chlorinated hydrocarbons, carbamates, organophosphates, pyrethroids and also juvenoids and benzoylphenylureas (BPUs).

2.2.1 Breeding procedure

M. domestica was reared under a constant temperature of 25°C. Larvae were fed with curd and 'Altromin'® in photoscales. Development took about six days. The puparia were collected and kept until hatching. For the test procedure, only three-day-old flies were used.

2.3 Biological tests

2.3.1 Filter test (adult flies)
Filter paper discs (diameter 9·5 cm) were treated with the test insecticide dissolved in acetone and kept in glass jars (diameter 10 cm, height 6 cm). Concentrations of 0·13 μg to 10 mg ml^{-1} active ingredient were tested in parallel. Ten flies, which were narcotised with carbon dioxide, were placed in each jar and mortality of the flies was monitored after defined time intervals up to 24 h.

2.3.2 Topical application
Adults of *M. domestica* were narcotised with carbon dioxide and each animal was treated topically, using an electronic microapplicator (Microlab P, Hamilton). Insecticides were dissolved in acetone and 1·0 μl of a given concentration was dropped on the mesonotum of the flies. The control flies were treated with the solvent only. Mortality was assessed after defined time intervals up to 24 h post-treatment.

2.3.3 Larvicidal test
Cattle manure was put into plastic jars and was sprayed with the test larvicide emulsified in water. Fifty 2nd-instar larvae were then placed on the manure and the jars were kept for three to four weeks, after which numbers of dead larvae, puparia and flies were recorded. The results were compared to controls with the solvent alone.

2.4 Enzyme assays

2.4.1 Preparation of microsomes
Microsome preparations were carried out according to Lee and Scott.[14] House flies were frozen for at least one day at −80°C. Heads, thoraxes and abdomens were detached by shaking the frozen flies on a metal sieve (mesh 2 mm). Abdomens were removed, placed on ice and 500 of these were transferred immediately into homogenisation medium (10 ml) consisting of sodium phosphate buffer (100 mM; pH 7·5) containing 1 mm phenylmethylsulfonyl fluoride (PMSF), 1 mM phenylthiourea (PTU, presolved in ethylene glycol monomethyl ether), 1 mM EDTA, 1 mM DL-Dithiothreitol (DTT), and glycerol, 100 ml $litre^{-1}$, and were homogenised for 30 s in a motor-driven glass-Teflon potter (Braun-Melsungen, Germany) at 1400 rev min^{-1}. The homogenate was filtered through four layers of cheesecloth, the residue was again homogenised with homogenisation medium (10 ml; 15 s) and then filtered through two layers of cheesecloth. The homogeniser was rinsed with homogenisation medium (5 ml) and all fractions were pooled and centrifuged for 20 min at 10000*g* at 4°C (Heraeus Varifuge K, fixed angle rotor 05180; Heraeus Instruments, Hanau, Germany). The supernatant was filtered through two layers of cheesecloth and centrifuged (1 h 100000*g*; 4°C) (Kontron ultracentrifuge, fixed angle rotor 70·38 (Kontron Instruments GmbH, Neufahrn, Germany). The pellet was resuspended in a resuspension medium (2 ml; sodium phosphate buffer, 100 mM; pH 7·5, containing 1 mM PMSF, 1 mM EDTA, 1 mM DTT and glycerol, 200 ml $litre^{-1}$) in a glass-Teflon potter homogeniser and diluted to a protein concentration of 2 mg ml^{-1}. Aliquots (2 ml) were stored in glass vials at −80°C for several days.

2.4.2 Determination of protein contents
Protein was determined by the method of Bradford,[15] using bovine serum albumin as a standard.

2.4.3 Determination of cytochrome b_5
The determination was performed essentially as described by Estabrook and Werringloer.[16] Difference spectra were measured in a dual beam spectrophotometer. Hexobarbital (10 μl) dissolved in methanol (final concentration 1 mM) was added to a microsomal suspension (Section 2.4.1) containing 1 mg protein ml^{-1}.[17] The suspension was divided between two cuvettes (sample and reference). The cytochrome b_5 concentration was determined by fully reducing the suspension in the sample cuvette with sodium dithionite (a few crystals) and recording the difference spectra between 400 and 460 nm. The change in absorbance at 426 nm and at 409 nm was determined. For calculation of the cytochrome b_5 concentration an extinction coefficient of 185 mM^{-1} cm^{-1} was applied.

2.4.4 Determination of cytochrome P-450
The determination was essentially performed as described by Estabrook and Werringloer.[16] The procedure was essentially that described in Section 2.4.3 except that the sample cuvette was aerated gently with carbon monoxide (1 min) and difference spectra between 390 nm and 510 nm were recorded. The change in absorbance at 450 nm and at 490 nm was determined. For calculation of the cytochrome P-450 concentration an extinction coefficient of 91 mM^{-1} cm^{-1} was applied.

2.4.5 Determination of acetylcholine esterase (AChE; EC 3.1.1.7) activity
2.4.5.1 Preparation of enzyme. M. domestica heads (adults, four days old; 1 g) were homogenised in sodium phosphate buffer (10 ml; 50 mM; pH 7·5) for 30 s in a motor-driven glass-Teflon potter at 1500 rev min^{-1} at 0°C. The homogenate was filtered through cheesecloth and the supernatant was centrifuged (15 min; 1500*g*).

TABLE 1
Response of Different Fly Strains to Acetylcholine Esterase Inhibitors

Compound	Fly strain	LC_{50} (mg AI m^{-2}) (±SE)	Resistance factor[a]
Propoxur	WHO-N	6·65 (±2·0)	—
	Hans	>3500	>530
	Reichswald	>3500	>530
	Grimm	>3500	>530
Trichlorfon	WHO-N	0·525 (±0·16)	—
	Hans	23·1 (±11·14)	40
	Reichswald	>3500	>6600
	Grimm	>3500	>6600
Fenitrothion	WHO-N	0·52 (±0·13)	—
	Hans	>3500	>6700
	Reichswald	220·5 (±29·4)	424
	Grimm	350 (±172)	673
Phoxim	WHO-N	0·665 (±0·24)	—
	Hans	>3500	>5000
	Reichswald	>3500	>5000
	Grimm	>3500	>5000

[a] LC_{50} 'Grimm'/LC_{50} 'WHO-N'.

2.4.5.2 Acetylcholine esterase activity. Activity in the resulting supernatant was determined by the method of Ellman *et al.* with minor modifications.[18] 5,5'-Dithio-*bis*-(2-nitrobenzoic acid) (250 μM; 1 ml) in sodium phosphate buffer (50 mM; pH 7·5) and acetylthiocholine (5 mM; 100 μl) were added to the enzyme preparation (100 μl). The reaction was followed by continuous measurement of absorbance at 405 nm in a spectrophotometer at room temperature. For calculation of the acetylcholine esterase activity an extinction coefficient for the thiophenolate (TP) ion of 13·3 mM^{-1} cm^{-1} was applied.

2.4.6 Determination of glutathione S-transferase (GST; EC 2.5.1.18) activity

2.4.6.1 Preparation of enzyme. Three- to five-day old adult *M. domestica* (2 g) were homogenised (30 s) in a solution (10 ml) containing 50 mM Tris-HCl pH 8·0, 100 mM sodium chloride, 1 mM, EDTA, in a motor driven glass-Teflon potter at 1500 rev min^{-1} at 4°C. The homogenate was centrifuged (5 min; 1000*g*; Heraeus Varifuge RF, Heraeus Instruments, Hanau, Germany), the supernatant then being centrifuged (15 min; 15000*g*) and the resulting supernatant also centrifuged (1 h; 126000*g*; 4°C). The supernatant from the final centrifugation was filtered through 0·45 μm Millex HA (Millipore, Germany).

2.4.6.2 Determination of glutathione S-transferase activity. This followed the method of Habig and Jakoby.[19] To an enzyme preparation (1–2 mg protein), L-glutathione (reduced form, 5·94 mM final concentration), Tris-HCl (50 mM) and EDTA (1 mM; pH 8·0) were added to a final volume of 1 ml and placed in a 1-ml cuvette. The reaction was started by addition of 1,2-dichloro-4-nitrobenzene (DCNB; 60 mM; 10 μl) in ethanol, and the increase in absorbance was followed by continuous measurement of absorbance over 1 min at 345 nm in a spectrophotometer at room temperature. A unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of 1 μmol *S*-2-chloro-4-nitro-phenylglutathione (CNPG) using 1 mM DCNB. Concentration of the product CNPG (*S*-2-chloro-4-nitro-phenylglutathione) was calculated from its extinction coefficient at 345 nm: $\varepsilon = 8{\cdot}5$ mM^{-1} cm^{-1}.

2.5 Statistical analysis

Probit-log transformations and statistical analysis of linear regressions were performed using Sigma Plot, Jandel Scientific.

TABLE 2
Response of Several Fly Strains to Cyfluthrin and beta-Cyfluthrin in a Filter Paper Assay

Compound	Fly strain	LC_{50} (mg AI m^{-2}) (±SE)	Resistance factor[a]
Cyfluthrin	WHO-N	0·30 (±0·095)	—
	Hans	20·3 (±16·8)	67·7
	Reichswald	73·5 (±13·6)	245
	Grimm	87·5 (±36·2)	292
Beta-Cyfluthrin	WHO-N	0·07 (±0·13)	—
	Hans	9·1 (±1·37)	130
	Reichswald	63 (±11·6)	900
	Grimm	20·3 (±3·13)	290

[a] LC_{50} 'Grimm'/LC_{50} 'WHO-N'.

TABLE 3
Response of the Multi-resistant Fly Strain 'Grimm' to the Isomers of Cyfluthrin in a Topical Application Test

Compound enantiomer pair	LC_{50} *(μg AI per fly)* (±*SE*)	*factor*[a]
Cyfluthrin	0·03 (±0·004)	
I (*cis*)	>2	<0·015
II (*cis*)	0·012 (±0·002)	2·5
III (*trans*)	>2	<0·015
IV (*trans*)	0·014 (±0·003)	2·14

[a] Efficacy of the isomer in comparison to cyfluthrin.

3 RESULTS

The classical treatment against flies, particularly in the 1950s and 1960s, involved propoxur, and this compound was used until resistance to carbamates developed. This insecticide, tested in the filter paper test against the susceptible 'WHO-N' strain had an LC_{50} value (concentration causing death of 50% of the adults in the test) of 6·71 mg AI m^{-2}, whereas the values for the resistant 'Hans', 'Reichswald' and 'Grimm' strains were all >3500 mg AI m^{-2}, giving a resistance factor >530 (Table 1). Against the susceptible 'WHO-N' strain, the organophosphates phoxim, fenitrothion and trichlorfon had LC_{50} values of 0·7, <0·2 and 0·5 mg AI m^{-2}, respectively, but the corresponding values for the resistant strains indicated resistance factors between 424 and >6700 for fenitrothion, 40–>6600 for trichlorfon and >5000 for phoxim (Table 1).

Cyfluthrin consists of four isomers each of which encompasses two enantiomers, these pairs of enantiomers being designated **I**, **II**, **III** and **IV**, respectively. Cyfluthrin contains all four enantiomeric pairs, whereas beta-cyfluthrin contains only two, pair **II** and pair **IV**. According to Gruning *et al.*[20] and Leicht *et al.*[21] the good insecticidal activity of cyfluthrin is caused predominantly by these two enantiomeric pairs.

The data in Table 2 show that cyfluthrin showed good activity (LC_{50} 0·3 mg AI m^{-2}) against the susceptible 'WHO-N' strain of *M. domestica* but poor efficacy against the multi-resistant strains. Beta-cyfluthrin gave similar results with the 'Hans' and 'Reichswald' strains but 10-fold better activity than cyfluthrin against the multi-resistant 'Grimm' strain.

Results with the individual enantiomer pairs against the multi-resistant 'Grimm' strain are given in Table 3. The vastly greater activities of pairs **II** and **IV** compared with those of pairs **I** and **III** explain the superior performance of beta-cyfluthrin over that of cyfluthrin when tested against the susceptible strain.

In order to gain information on the resistance mechanisms, synergists were used, such as PBO (mixed function oxidase, MFO), DEF (esterases) and Cibacron blue

TABLE 4
Response of Two Fly Strains to Cyfluthrin/Synergist Combinations in Filter Paper Assay

Insecticide	*Synergist*	*Fly strain*	LC_{50} *(mg AI* m^{-2}*)* (±*SE*)	*Resistance factor*[a]	*Synergism factor*[b]
Cyfluthrin	None	WHO-N	0·30 (±0·096)	—	
		Grimm	304·5 (±56·2)	1014	
None	PBO	WHO-N	1820 (±263·6)	—	
		Grimm	6055 (±1679)	3·3	
None	Cibacron blue[c]	WHO-N	>1400	n.c.[d]	
		Grimm	>1400	n.c.	
None	DEF	WHO-N	1820 (±404)	—	
		Grimm	>35000	>19	
None	Coumaphos	WHO-N	25·2 (±8·87)	—	
		Grimm	>35000	>1389	
Cyfluthrin	PBO	WHO-N	0·14	—	1·4
		Grimm	9·1 (±2·24)	65	33·4
Cyfluthrin	Cibacron blue[c]	WHO-N	0·56 (±0·10)	—	0·5
		Grimm	24·85 (±6·39)	44·4	12·3
Cyfluthrin	DEF	WHO-N	0·88 (±0·23)	—	0·3
		Grimm	31·85 (±6·23)	36·2	9·6
Cyfluthrin	Coumaphos	WHO-N	1·4 (±0·35)	—	0·2
		Grimm	101·5 (±14·26)	72·5	3

[a] LC_{50} 'Grimm'/LC_{50} 'WHO-N'.
[b] LC_{50} cyfluthrin/LC_{50} cyfluthrin + synergist.
[c] Limit of solubility in acetone was 3·2 mg ml^{-1}.
[d] n.c.: not calculated.

TABLE 5
Response of Larvae of Two Fly Strains to Insect Growth Regulators with or without Synergists

Insect growth regulator	*Synergist*	*Fly strain*	LC_{50} (*mg AI* m^{-2}) (±*SE*)	*Resistance factor*[a]	*Synergism factor*[b]
Triflumuron	None	WHO-N	1·9 (±0·28)	—	n.c.[d]
		Grimm	>10000	~15000	n.c.
Pyriproxyfen	None	WHO-N	0·52 (0·08)	—	n.c.
		Grimm	27·57 (±5·63)	53	n.c.
Cyromazine	None	WHO-N	1·58 (±0·38)	—	n.c.
		Grimm	2·75 (±0·56)	174	n.c.
None	PBO	Grimm	>3000	n.c.	n.c.
	DEF		>3000	n.c.	n.c.
	Cibacron blue[c]		>3000	n.c.	n.c.
Triflumuron	PBO	Grimm	>10000	~500	~1
	DEF		691·83 (±375)	364	>14
	Cibacron blue[c]		≫300	≫160	≪33
Pyriproxyfen	PBO	Grimm	63·09 (±4·72)	121	0·44
	DEF		109·64 (±11·27)	211	0·25
	Cibacron blue[c]		36·3 (±4·77)	70	0·76

[a] LC_{50} 'Grimm'/LC_{50} 'WHO-N'.
[b] LC_{50} cyfluthrin/LC_{50} cyfluthrin + synergists.
[c] Limit of solubility in acetone was 3·2 mg ml^{-1}.
[d] n.c.: not calculated.

(glutathion-*S*-transferase), known to block important resistance mechanisms preferentially. Cyfluthrin mixed with different synergists in the ratio 1 : 10 was used to elicit information on the mode of resistance against this pyrethroid. As shown in Table 4, synergists alone were active against susceptible or multi-resistant flies only at the very high concentration of at least 1 g m^{-2}. However, when applied together with cyfluthrin, all synergists were able to restore at least some of the activity of the insecticide. Synergistic factors of the multi-resistant 'Grimm' strain for PBO, DEF and Cibacron blue of 33·4, 9·6 and 12·3, respectively were calculated from results in Table 4.

The compound coumaphos displays non-specific esterase inhibition.[2] When tested with cyfluthrin against flies ('WHO-N', 'Grimm'), only weak synergistic effects were observed. In addition, coumaphos, when applied alone against 'Grimm', totally failed to control this strain. All synergists were used at concentrations clearly below the 'insecticidal threshold' ($LC_{50} \times 10^{-1}$) of the synergist.

From the strains investigated, only 'Grimm' has been treated with insect growth regulators, e.g. benzoylphenylureas (BPUs), thus far. Therefore, this strain was used for further investigations on resistance against larvicides (Table 5).

Triflumuron had an LC_{50} value of 1·9 mg m^{-2} against the susceptible *M. domestica* strain 'WHO-N' but did not prevent larval development in the multi-resistant strain 'Grimm', even at >10000 mg AI m^{-2}.

TABLE 6
Concentrations and Activities of Different Enzymes from a Resistant and a Susceptible House Fly Strain

		Musca domestica *strain*		
Enzyme	*Unit*	*'WHO-N'*	*'Grimm'*	*Factor*[a]
GST	μmole CNPG min^{-1} mg^{-1} (±S.E.)	0·159 (±0·019)	0·561 (±0·037)	3·53
AChE	nmol TP min^{-1} mg^{-1} (±S.E.)	6·778 (±0·490)	6·811 (±0·580)	1·00
Cytochrome b_5	nmole mg^{-1} (±S.E.)	0·119 (±0·016)	0·157 (±0·019)	1·32
Cytochrome P-450	nmole mg^{-1} (±S.E.)	0·231 (±0.035)	0·522 (±0·147)	2·26

[a] Resistant/susceptible.

Similar results were also obtained for other BPUs (diflubenzuron, flufenoxuron and lufenuron; data not shown).

Although no juvenoids have been applied to 'Grimm', a pronounced resistance to the juvenoid pyriproxyfen has been observed. A resistance factor of 53 was calculated.[22]

The synergists PBO, DEF and Cibacron blue, when applied alone, had no effect on the larval development up to the highest tested dose (3000 mg AI m^{-2}) (Table 5). Of all synergists tested against 'Grimm', only DEF could synergise the efficacy of triflumuron (factor of synergism >14). The combined application of pyriproxyfen and synergists slightly reduced the efficacy of this juvenoid.

Only cyromazine, a larvicide which interferes with sclerotisation processes during moulting and pupation,[23,24] was able to control 'Grimm' development efficiently (resistance factor 1·74).

Enzymes known to play an important role for the development of insecticide resistance are GST, AChE and MFOs. Therefore the activity of these enzymes was determined in a comparative study of the susceptible 'WHO-N' and the multiresistant 'Grimm' strains (Table 6). The concentration of the cytochrome b_5 and the activity of AChE were not significantly altered in the resistant strain. In contrast, the activity of GST was 3·5-fold higher in 'Grimm' homogenates and the concentration of cytochrome P-450 was about 2·3-fold higher in 'Grimm' microsomal preparations.

4 DISCUSSION

From 1987 to 1995 more than 30 *M. domestica* strains from livestock farms from the lower Rhine district were tested, using the methods described above, for their resistance levels in comparison to field strains from the area around Cologne, Wuppertal and Upper Franconia (Bavaria), with similar results. There was always a pronounced resistance against carbamates, organophosphates and pyrethroids.[7] Similar results for the house fly were obtained in Denmark by Keiding and Jespersen.[2]

Resistance against inhibitors of acetylcholine esterase (AChE) has also been described for different organophosphates and carbamates in various arthropod species[8] and for pyrethroids, which were shown to affect voltage-gated sodium channels, particularly in arthropods.[25] Resistance within a defined strain of *M. domestica* often results from a combination of different defence mechanisms rather than exclusively from an induction of one type.[7]

As shown in Table 1, all inhibitors of AChE tested failed to control any of the multi-resistant strains. Since not all of the tested compounds have been applied to the fly strains previously, it can be concluded that common resistance mechanisms against AChE inhibitors exist, regardless of the structure of the applied compounds. Comparable observations have been made for other *M. domestica* and *Blattella germanica* L. strains, assuming the existence of several modes of resistance mechanisms.[8,26] The enzyme activities of the tested strains suggest that target site resistance to inhibitors of AChE is of only minor importance.[8] Similar observations have been described for cockroaches[26] and for the moth *Platynota idaeusalis* Walk.[27] The pyrethroids known to act on voltage-gated sodium channels in the axonal membranes have been used for about twenty years for fly control.[12] Therefore cyfluthrin and the recently launched beta-cyfluthrin were tested for their resistance-breaking activity in the above-mentioned strains. All three field strains showed a significant resistance against these pyrethroids. Cyfluthrin was about twice as active as beta-cyfluthrin against 'Hans', whereas 'Reichswald' flies were about four times more susceptible to cyfluthrin than to beta-cyfluthrin. Resistance-conferring enzymes might not be able to differentiate between biologically active and inactive isomers of cyfluthrin. The inactive isomers might compete with the active isomers at assumed substrate binding sites of metabolic enzymes. Similar conclusions have been drawn for different resistant *Plutella xylostella* L. strains, *Helicoverpa armigera* Huebn. and *Heliothis virescens* F.[21,28,29] It is speculated that these phenomena are due to the fact that in pyrethroids containing a high content of *trans* isomers, such as cyfluthrin, beta-cyfluthrin or cypermethrin, these inactive isomers interfere with detoxification reactions conferring resistance.[30,31] Furthermore, this indicates that target-site resistance may not be predominantly responsible for the resistance observed in the 'Hans' and 'Reichswald' strains. 'Grimm' was more susceptible to beta-cyfluthrin than to cyfluthrin, which might be explained by the higher concentration of active isomers in beta-cyfluthrin.[21] As shown in Table 3, this could also be confirmed in topical application studies. Isomers **II** and **IV** are the most active ingredients of cyfluthrin, as shown in Table 3. Similar results were reported for *Dysdercus albofasciatus* Berg. and various other arthropods.[21,32]

At high concentrations, isomers **I** and **III** displayed some activity against the fully susceptible 'WHO-N' strain. This effect might be explained by an isomerisation of **I** and **III** to the active isomers **II** and **IV** taking place during the relatively long incubation period.[21] Such an isomerisation was observed previously after incubation of inactive isomers **I** and **III** in methanol in bioassays with *Daphnia magna* Straus.[25] Consequently, if detoxification significantly contributes to resistance in the 'Grimm' strain, synergists should restore sensitivity to the insecticides used, at least to some extent. This assumption could be confirmed (Table 4), but synergists showed different activities.

PBO had a three-fold higher synergistic activity than Cibacron blue and DEF, which both enhanced the susceptibility about 10-fold. This indicates that MFOs play a predominant role in the detoxification mechanisms of 'Grimm'. The importance of MFOs in detoxification has also been described for the house fly.[12,33]

Especially in multi-resistant strains of *Musca domestica*, it has been reported that more than one mechanism contributes to the level of resistance.[28] GST and hydrolases, as well as MFOs, are often found to be responsible for metabolic resistance.[10] As shown in Table 6, enhanced levels of GST and cytochrome P-450 were present in the 'Grimm' strain, suggesting the significance of these enzymes in the resistance mechanisms of 'Grimm'.

Insect growth regulators such as triflumuron (chitin synthesis inhibitor), pyriproxyfen (juvenile hormone analogue; juvenoid) or cyromazine (antisclerotisation, cuticle synthesis inhibitor) display completely different modes of action. Therefore it was interesting to evaluate to what extent cross-resistance between different IGRs interferes with the activity of typical larvicides. The 'Grimm' strain has been treated often with BPU since 1988. This explains the high level of resistance against triflumuron (Table 5). Resistance in *M. domestica* against diflubenzuron, another benzoylphenylurea has been reported to be primarily based on an enhanced esterolytic activity.[34] This is in accordance with our results from combinations of triflumuron with synergists. Addition of DEF resulted in the highest suppression of resistance. Oxidative mechanisms in BPU detoxification seem to be less important. Interestingly, cyromazine was almost unaffected by the resistance mechanisms. Triflumuron did not induce specific defence mechanisms against cyromazine, though cyromazine resistance has been reported.[35,36]

In contrast to cyromazine, a cross-resistance to pyriproxyfen could be determined in the multi-resistant 'Grimm' strain. Similar results for cross-resistance with DDT, pyrethroids and juvenile hormone analogues were described in *M. domestica*.[37] Combinations of pyriproxyfen with any of the synergists did not improve the efficacy of this compound.

Our results indicate that specific information about the resistance status of house fly populations is important for the development of fly control programmes and is a prerequisite to successful application of combinations of synergists and insecticides.

The enzymes GST, AChE and MFOs are known to play an important role in the development of insecticide resistance. Therefore the activity of these enzymes in the susceptible 'WHO-N' and the multi-resistant 'Grimm' strains was compared (Table 6). The concentration of the cytochrome b_5 and the activity of AChE were not significantly different in the two strains but, in contrast, the activity of GST was 3·5-fold greater in 'Grimm' homogenates and the concentration of cytochrome P-450 was about 2·3-fold higher in 'Grimm' microsomal preparations.

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